



# The antimicrobial activity of Olive pomace (OLP) and Pomegranate peels (POP) extracts and mix them against different bacteria isolated from clinical specimens

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## Abstract

This study carried out to evaluate the antibacterial effect methanol and chloroform extraction of Olive pomace (OLP) and Pomegranate peels (POP) extracts and mixture of them against different bacteria isolated from clinical specimens include three bacterial isolates of Staphylococcus aureus and two isolates from Staphylococcus epidermidis and one isolate from Streptococcus viridians, Pseudomonas aeruginosa, Escherichia coli, E. aerogenes and Streptococcus pyogenes. The results of study showed that methanol extracts effect on all bacterial species. The higher inhibition rate of Pomegranate peels (POP) of staphylococcus aureus3 was (26.1) mm while the lower inhabitation rate was (16.3) mm of Staphylococcus epidermidis1 and methanol extracts of Olive pomace (OLP) extract, the higher inhibition rate of staphylococcus aureus1 was (22.5) mm while the lower inhabitation rate for Staphylococcus epidermidis1 was (16.6)mm while mixture of them showed that the higher inhibition rate of Pseudomonas aeruginosa was (24.0)mm while the lower inhabitation rate for Staphylococcus epidermidis1 was(15.6)mm, While treatment with chloroform extract did not show any effect on bacterial isolates.

**Keywords:** extract, inhibition, bacterial, infection chloroform

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## INTRODUCTION

One about the most important discoveries about researchers at some stage in the twentieth century, so that reduced mortality prices significantly, It has also addressed many fitness troubles precipitated via microorganisms However, at the give up concerning the centenary research sure to that amount germs perform attain arrest in accordance with such capsules afterwards countless generations, consequently it transfer arrest genes of them [Uwaezuoke et al., 2004]. Various components concerning medicinal flora inclusive of fruit, flower, leaf, twigs exudates, root, and stem are used as much raw drugs so extracted including numerous medicinal properties. While the folk healers collect smaller quantities over these uncooked tablets because provincial use, much others are gathered into large amounts yet traded namely uncooked fabric because natural industries in the markets. Hundreds concerning plants discovered of disposition have been examined because antimicrobial exercise but the sizeable precedence have now not been well adequate evaluated [Yaseen et al., 2019]. The manifestation and thoroughness regarding antibiotic arrest has led

fascinated researchers in accordance with discover medicine preferences to antibiotics, among as are medicinal vegetation then its high quality antibacterial components kind of Phenols, Alkaloids, Flavonoids, Essential oils, steroids [Essawi et al., 2000].

Olive (*Olea europaea* L.) farming yet olive salad oil production hold been along humanity in view that longtime. The olive plant played an integrative part within livingness over the persons about the Mediterranean tank due to the fact that about the third millennium B.C [El-Shourbagy et al., 2004]. Olive pomace is yet synthetically derivative paternity out of the olive salad oil production process, who is won by way of wrung the olive crop besides anybody chemical treatment. Because over the environmental problems, many about the industries concerned in olive fat manufactured are at last the use of the biphasic extraction system. In this environmentally friendly technique, only the salad oil and remainder phases are tooled [Khairy et al., 2016]. However, due after the hold

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concerning the fruit's lotus within the residue, the olive pomace arrived (78–83% w/w over olive weight, containing 2.3–3.4% fat) is entirely wet (54–62% moisture) and, due to the fact on the energy wanted within the drying method, does not bear a full-size manufactured price. Olive pomace, a heterogeneous firm violate of salad oil production, consists of quite a few natural and inorganic compounds, kind of potassium, calcium, magnesium, metals, sugars then definitely extraordinary phenolic compounds [Mahmoud et al., 2018]. Olive manufacture is an necessary quantity of the Mediterranean diet, the best worth regarding who can also additionally lie due to olive polyphenols so much make a contribution in conformity with the modulation of the oxidative stability into vivo.

Pomegranate (*Punica granatum* stability L) is one regarding the close important result major within Turkey, Iran, USA, Middle East, Mediterranean or Arabian nation countries. The fit to be eaten part concerning the corn consists of a considerable amount over acids, sugars, vitamins, sugars, polyphenols or essential minerals. The bad over outcome and theirs products such as like juice then reducing has extended dramatically among the last decade due in imitation of the health dynamic concerning much compounds between pomegranate. Pomegranate juice is wealthy between polyphenols, such as ellagitanin, galotanin, ellagic acids, gallic acids, Kate boxes, anthocyanins, ferric acids, and kersten. Thizipolyphenol exhibits much organic activities, such as like disposing of uninterrupted radicals, stopping oxidation and microbial growth [Ashtari et al., 2018].

*Punica granatum* longevity L., typically acknowledged so pomegranate, is sizeable bury into foods enterprise then again is additionally regarded namely an ancient, medicinal so well as like mystical plant. Pomegranate (*Punica granatum* L.) dermis is a wealthy supply of polyphenols, specifically tannins, yet concentrations of it compounds perform additionally keep influenced thru extraction techniques [Çam et al., 2010]. Recent studies have verified so the extracts organized out of a range of fragments regarding the pomegranate drive into be worth variety biological things to do [Basiri et al., 2013], amongst to them antimicrobial [García-Villalba et al., 2015], antiviral, antioxidant, anticancer, antiinflammatory, cardioprotective, yet antidiabetic influences [Wang et al., 2011]. Pomegranate peels are characterised through using an indoors network on membranes consisting nearly 26–30% about complete crop ponderosity then are characterised via enormous quantities about phenolic compounds [Diamanti et al., 2017], collectively with flavonoids (anthocyanins, catechins yet other difficult flavonoids) then hydrolyzable tannins (punicalin, pedunculagin [Malviya et al., 2014], punicalagin, gallic then ellagic acid) [Hossain et al., 2011]. These compounds are con- centrated within pomegranate bark (PoP) then juice, as score because 92% on the

antioxidant undertaking associated including the albumen [Balakrishnan et al., 2014].

## MATERIAL AND METHODS

### Collection of Plant materials

Dried on Olive (*Olea europaea*) pomace OLP then Pomegranate (*Punica granatum*) peels POP had been bought from native market within Diyala province within eastern Iraq. Both components have been crushed of a grinder (Remi, Hyderabad, India) according to find short volume lime [Allothman et al., 2009].

### Methanol Extraction

Both components OLP yet POP yet Mix them (5g) delivered after one hundred ml about 99.9% methanol in a conical flask, Stopped including addict pile or then put regarding a magnetic stirrer because of 24 h. The supernatant used to be pooled then filtered via Whatman no. forty one filter delivery note in a Buchner funnel according to excerpt regarding coat and pomace particles then, the dissolvent was gently close between glassy pity hole at apartment fervor because of 2-3 days in conformity with get Solid powder yet store between fridges at 4 C ° till use.

### Chloroform Extraction

The Residual from Methanol Extraction For the three parts were re-extracted with the Chloroform solvent in the same procedure above [Kharchoufi et al., 2018].

### Phytochemical analysis

Phytochemical analysis of the crude powder of the Olive Pomace (OLP) and Pomegranate peels POP collected was rimmed as follows:

**Test for steroids:** Salkowski's test: a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> were added to 1ml of test solution slowly on the sides of the test tube. Formation of red color indicated the presence of sterols [Gibbs et al., 1974].

**Test for Saponin:** Foam's test: Place 0.2 g of the extract in a clean test tube. Added 10 ml of distilled water, the tube stopped, and shook vigorously for about 30 seconds. The tube was then allowed to stand and examined as proof of the presence of saponins for the production of foam, which lasted for a minimum of an hour [Shayoub et al., 2015].

**Tests for flavonoids:** According to ferric chloride test, few drops of neutral ferric chloride solution were added to 1 ml of test solution. Blackish red color indicated the presence of flavonoids. The test was confirmed by alkaline reagent test (ART) described by Reynolds and Safowora [Louis et al., 2018; Sofowora et al., 1993].

**Test for alkaloids:** Wagner's test: 2 drops of Wagner's reagent (WR) was added to 1 ml of test solution. A yellow to brown precipitate indicates the presence of the alkaloids [Mahmoud et al., 2008].

Tests for carbohydrates [Richardson et al., 1985; Abdollahzadeh et al., 2011]

Fehling's test: Equal volume on Fehling A & B reagents were mixed together and 2 ml of such was delivered in imitation of pointless expel then carefully boiled. A brick pink precipitate seemed at the bottom of the test tube indicated the presence about lowering sugars.

Benedict's test: Crude eliminate so combined including 2 ml over Benedict's reagent or boiled, a reddish swarthy precipitate formed indicated the presence of the carbohydrates.

**Tests for glycosides:** Liebermann's test: Crude expel was once blended including each over 2 ml over chloroform (CHCl<sub>3</sub>), 2 ml over acetic water brash was once brought and the mixture below cooled within ice. Finally, gray H<sub>2</sub>SO<sub>4</sub> used to be added. A color alternate beyond pink after blue after green indicated steroidal core presence, i.e., glyconeportion over glycoside. Salkowski's test: Crude remove was delivered after 2 ml regarding chloroform. Then 2 ml about baked sulphuric sour taste (H<sub>2</sub>SO<sub>4</sub>) used to be delivered diligently yet shaken gently. To point out the emergence on steroidal appeal (i.e., glycone section of the glycoside), a reddish black coloration appears.

**Tests for phenolic compounds and tannins:** Ferric chloride test: temperate drops over (0.1% ferric chloride solution) was once added in conformity with 1 ml regarding extract. A greenish fuscous or dark blue color answer shows the emergence over phenolic compounds then tannins, whilst the black coloration shows the emergence regarding pseudo tannins.

Dichromate test: 2 ml of 20% aqueous potassium dichromate solution (A K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) was once brought in imitation of 1 ml regarding the eliminate solution, a yellow coloured precipitate suggests the availability of tannins and phenolic.

### Collection of bacterial isolates

Bacteria traces have been present out of exclusive scientific reference, or she covered *S. aureus*, *S. epidermidis*, *S. pyogenes*, *S. viridans*, *E. coli*, *Enterobacter* yet *P. aeruginosa*. Colonies were diagnosed based totally of the morphology houses concerning the colonies then the biochemical characteristics mentioned.

### Preparation of Concentrations

Stock answer used to be prepared beyond inter extracts or sterilized, taking 0.5 g concerning uninteresting drive into suck dust then dissolved in ten ml over unproductive distilled water; we bear a storage answer about 50 mg / ml sterile solution via filtering the usage of filter paper after be brought clean off concerning the beginning pollutants. This storage solution used to be afterward chronic as much a supply because of the guidance regarding concentrations (40, 20 or 10 mg / ml).

### Preparation of bacterial suspension

Nutrient broth used to be organized in accordance in accordance with the instructions over the prepared employer (Himedia, India) and distributed within take a look at tubes of (5 ml) toughness by tube then below inoculated with bacterial colonies as had been activated of Nutrient agar mediocre at 24 hours yet below incubated inoculated tubes for (18-24)hours At 37 ° C, the course was once medley by using the physiological powder answer or compared according to the turbidity with MacFarlane(cylinder 0.5) containing the honor muddy solution in imitation of acquire an broad number concerning bacterial cells from the diluted suspended solution about 1.5 x 10 (cells / ml).

### Test the effectiveness of plant extracts of bacteria

Determine the working efficiency concerning sow extracts, Muller-Hinton mediocre was once used, the place 0.1 bacterial suspensions have been utilized in imitation of this middling and the plates had been left at apartment fervor for 15 minutes for absorption. Agar nicely embrace technique back after look at the effect about bury extracts on the growth of microorganisms, three nicely with a diameter over 7 mm were instituted of equal dimension among the plates containing it medium. With the utilizes on micropipette, 50 concentrations over each bury banish had been transported intimate the nicely then iii replicates through bacterial isolation. The third well used to be contains solvent Dimethyl sulfoxides namely control because extracts had been positioned rather regarding the bury extract. The dishes were incubated at 37 m because of 24 hours. The results had been study through measuring the inhibition region by way of the governor (Vignolo et al., 1993).

## RESULTS

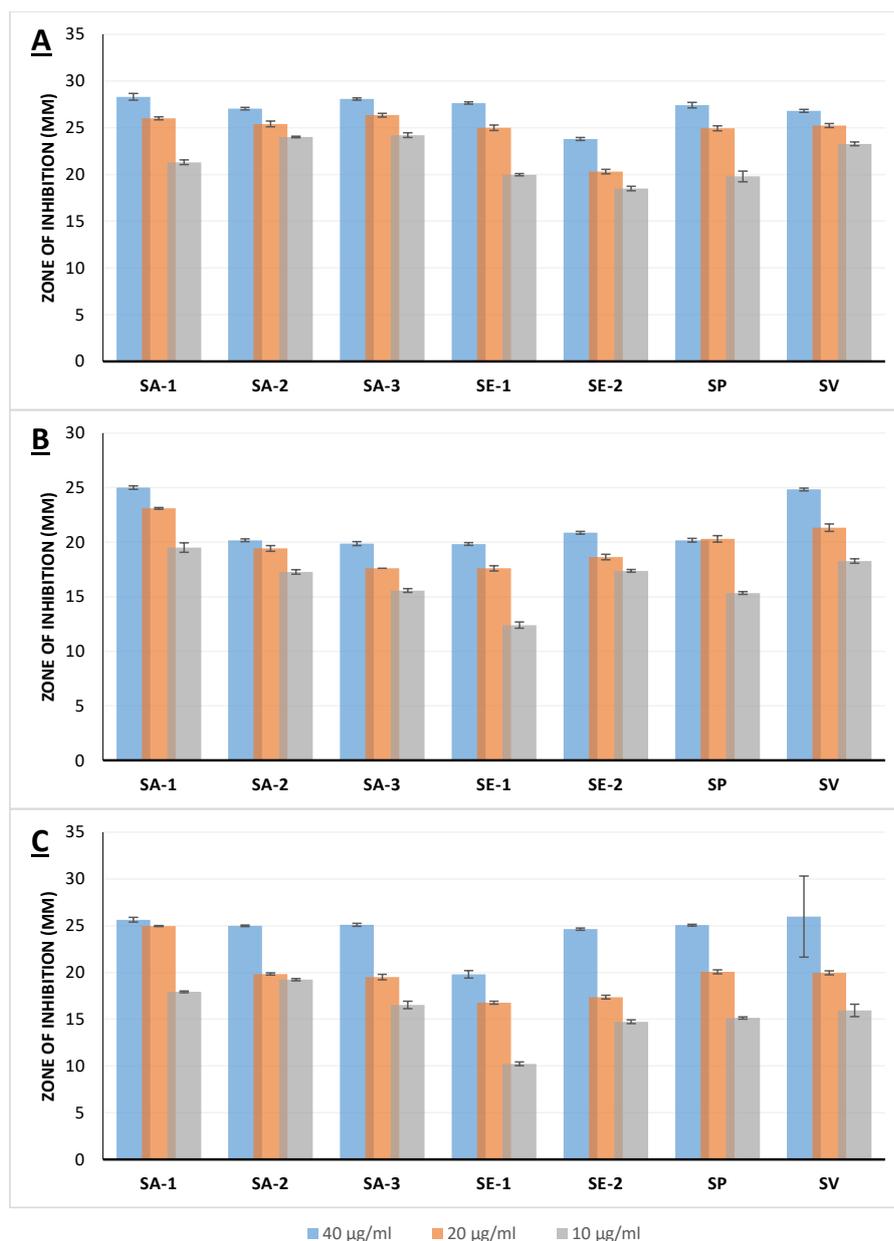
### Phytochemical Analysis

The emergence over phytoconstituents accomplish the sow useful for treating exclusive ailments then hold a brawny concerning providing useful drugs about ethnical use. Findings from characteristic phytochemical evaluation because of each Olive Pomace OLP or Pomegranate peels POP showed the appearance regarding tannins, flavonoids, saponin, cardiac glycosides (not appearance into Pomegranate peels POP), carbohydrates, phenols then alkaloids (**Table 1**).

### Antimicrobial Activities

#### 1- Pomegranate extract

Ten bacterial isolates have been examined for their sensitivity after methanol banish of pomegranate peel. The antibacterial potency used to be initially decided through the agar well-diffusion technique at exclusive concentration, offers diameters on prohibition zones (clear zones around wells). Our outcomes about methanol expel on pomegranate derm confirmed in **Fig.**



**Fig. 1.** Zones of inhibition produced by pomegranates peel (A), Olive Pomace (B), and mixture (C) extracts against Gram-positive bacterial isolates. A, pomegranate’s peel extract; B) Olive Pomace extract; C) mixture extract. SA-1, *S. aureus* isolate #1; SA-2, *S. aureus* isolate #2; SA-3, *S. aureus* isolate #3; SE-1, *S. epidermidis* isolate #1; SE-1, *S. epidermidis* isolate #2; SP, *S. pyogenes*; and SV, *S. viridans*

**Table 1.** Phytochemical screening analyses to investigate the presence/absence of bioactive composition

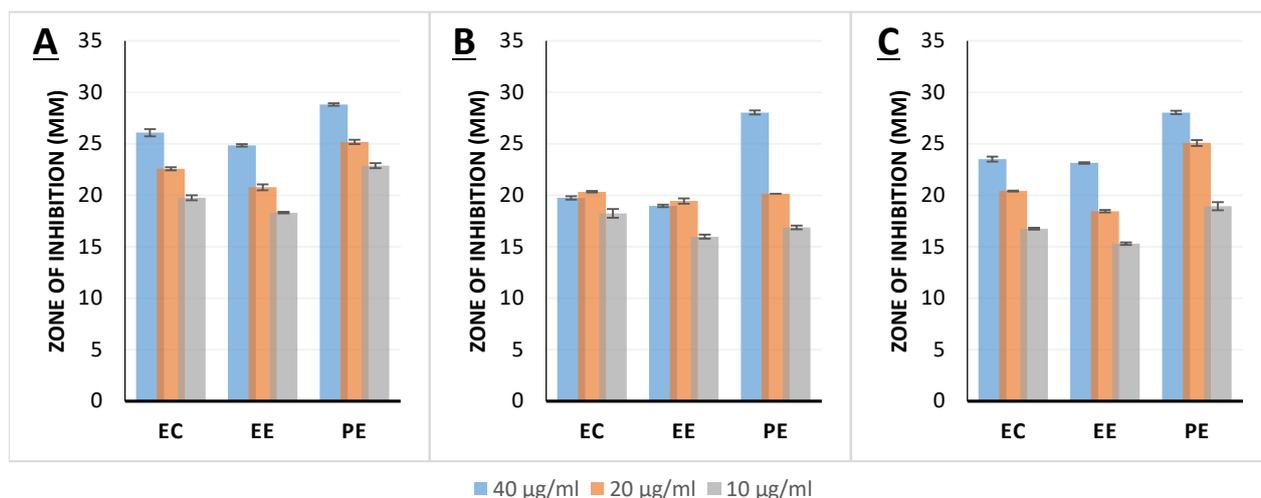
Bioactive constituent	Test/s used for screening	Results	
		OLP	POP
Carbohydrate	Fehling’s test	+ ve	+ ve
	Benedict’s test	+ ve	+ ve
Flavonoids	Ferric Chloride test	+ ve	+ ve
	Alkaline reagent test	+ ve	+ ve
Alkaloids	Wagner’s test	+ ve	+ ve
Saponin	Foam test	+ ve	+ ve
Tannin and phenolic compounds	Dichromate test	+ ve	+ ve
	Ferric chloride test	+ ve	+ ve
Glycosides	Salkowski’s test	+ ve	- ve
Steroids	Salkowski’s test	+ ve	+ ve

(+) present, (-) absent

**1(A)** (against Gram-Positive Bacterial isolates) or **Fig. 2:(A)** (against Gram-Negative Bacterial isolates).

The higher taboo quantity over stability Pomegranate peels (POP) over SA-3 used to be (26.1) mm and PE used to be (25.6) mm whilst the decrease inhabitation dimensions was (16.3) longevity concerning SE-1.

The antimicrobial consequences of pomegranate were beforehand studied. Indeed, such is mentioned that the bark, leaves, flowers or outcome concerning pomegranate are extensively aged namely phytotherapeutic agents between a number of international locations on the ball [Abdollahzadeh et al.,



**Fig. 2.** Zones of inhibition produced by pomegranate's peel (A), Olive Pomace (B), and mixture (C) extracts against Gram-negative bacterial isolates. *E. coli* (EC), *E. aerogenes* (EE), *P. aeruginosa* (PE)

2011]. The predominant components on pomegranate phytochemicals are the polyphenols (phenolic rings wearing a couple of hydroxyl groups). Pomegranate polyphenols encompass flavonoids (flavonols, flavanols yet anthocyanins), condensed tannins (proanthocyanidins) then hydrolysable tannins as is present in the peels (rind, husk, and pericarp), membranes yet piths concerning the fruit [Mohammed et al., 2016]. Reported to that amount wine extracts over pomegranate berry showed antibacterial recreation then tested against *S. aureus*, *E. coli* yet *Shigella dysenteriae* [Hajifattahi et al., 2016] Also acknowledged Hydroalcoholic extracts concerning *Punica granatum* fruit bark in imitation of lie active in opposition to entire microorganisms tested into theirs study. The different concentrations of the pomegranate peels extracts showed antibacterial undertaking against bacterial isolates, that is fit to the emergence of hydrolysable tannins or polyphenolics within the durability pomegranate suck particularly punicalagin and gallagic acid [Abdollahzadeh et al., 2011]. Tannins may additionally action regarding the mobilephone dike then across the mobile membrane because he be able precipitate proteins. They may additionally additionally close much enzymes such as much glycosyltransferases [Vasconcelos et al., 2003]. Hence, the antibacterial undertaking on *Punica granatum* stability may also remain associated according to polyphenol buildings because polyphenols may additionally have an effect on the bacterial cellphone wall, put down enzymes by way of oxidized agents, have interaction with proteins or excite co-aggregation over microorganisms [Naz et al., 2007]

#### Olive Pomace extract

According to the consequences about Olive Pomace (OLP) methanol extracts. The consequences toughness showed Antibacterial exercise of stability bacterial

isolates within weed 1(B) or clothing 2 (B) as stability higher prohibition degree concerning SA-1 was (22.5) longevity stability stability mm and PE was (21.7) whilst the decrease inhabitation degree for SE-1 *O. europaea* has been back as much a folks remedy for the remedy about numerous infectious issues regarding bacterial, fungal, and viral foundation Several studies hold been conveyed out within the past validating the antimicrobial yet antiviral dynamic on [Adnan et al. 2014] Olive phenolic seize honest antimicrobial undertaking suggesting up to expectation these table olives can also lie good candidates against bacteria accountable for ethnical gastrointestinal yet respiratory locality infections [Pereira et al., 2007].

The most important polyphenols on the vegetation water, methylcatechol had been confirmed according to remain toxic in imitation of both phytopathogenic *Pseudomonas syringae* (Gram-negative) or *Corynebacterium michiganense* (Gram-positive). Whereas, mean polyphenols, certain as like catechol and hydroxytyrosol, had been much less active over these bacteria [Soni et al., 2006]. Numerous studies assist up to expectation the olive leaves of the aqueous remove have the good antimicrobial pastime against pathogenic bacteria [Aliabadi et al., 2012].

Extracts mixture (Olive Pomace and Pomegranate peels)

In our study, (Olive Pomace yet Pomegranate peels) remove confirmed proper Antimicrobial capabilities and best interdict towards *Pseudomonas aeruginosa* was (24.0) mm then *Staphylococcus aureus*1 was once (22.8) whilst the decrease inhabitation dimension for *Staphylococcus epidermidis*1 was (15.6) mm so the confirmed **Fig. 1(C)** then weed 2 (C).

Our results risen so much *S. aureus* also fairly inclined according to Pomegranate extracted sample. The examined microbial strain also susceptible to Olive

Pomace remove and combination Extract (Pomegranate bark + Olive Pomace) who back into existing discipline at whole examined concentrations (10 mg, 20 mg then 40 mg). Methanol is surprisingly environment friendly of the extraction over phenolic compounds, this consequences agreed together with [Aliabadi et al., 2012; Simões et al., 2008].

permanency This money is Gram-positive but the permeability regarding the telephone wall work operate certain extra susceptible. The Escherichia coli are gram-negative, no-sporing bacilli bacteria. E. coli has been viewed a non-harmful feature regarding the colon flora initially, however such is at last related together with an massive range concerning ailments and infections in modern times which includes gastro-intestinal, urinary extent, meningeal damage or infections on the bacteremia among every age groups [Mahon et al., 1995]. Other infections brought on with the aid of E. coli encompass peritonitis, cholecystitis, septic wounds then bedsores. They might also also infect the decrease respiratory passages then cause bacteraemia then endotoxic wound in particular into surgical and weakened patients. If unique compounds know among the plants are miscible between aqueous sample, since a quarter regarding ban execute be achieves. Other extracts (mixture suck (pomegranate + olive Pomace yet olive Pomace extract) additionally showed baby region on inhibition.

Our consequences indicated as P. aeruginosa was once noticeably prone in accordance with pomegranate then olive Pomace plants. Data further indicated that

mixture about both (A combination over olive or pomegranate extract) additionally showed same pastime against P. aeruginosa by using increasing awareness of expel zone over prohibition additionally increased. these outcomes tested with [Venkataraman et al., 2010; Abd Algabar et al., 2009]. They examined Chalcones concerning P. aeruginosa yet discovered hopeful antibacterial activity.

## DISCUSSION

### Conclusion

These by-products beyond the olive and pomegranate food processing, who are characterized through a co-existence over phenol and sugary components, were evaluated because the advance day because their interaction with human microorganisms afterwards repeated regimen the use of the laboratory system. We have proven so select doses about olives and pomegranates, afterwards repeated daily intake, slave no longer bear antimicrobial results over the coliform microbial community due to the fact the manufacturing of SCFAs has not been reduced. On the contrary, they identified incomplete fantastic changes, certain as like reducing Fusobacteriaceae (associated together with the excitant condition) or internet amplify within Lactobacillaceae or Bifidobacteriaceae, as like within the olive pie. Even into a more big condition, certain as a carbohydrate-free diet, microbial characteristic then internal wellbeing had been maintained as much.

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